Genes for Tryptophan Biosynthesis in the Halophilic Archaebacterium *Haloferax volcanii*: the *trpDFEG* Cluster

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Tryptophan auxotrophs of the archaebacterium *Haloferax volcanii* define a cluster of overlapping genes homologous to eubacterial-eukaryotic *trpD*, -F, -E, and -G, linked in that order and each preceded by a possible ribosome binding site. Residues involved in feedback inhibition of eubacterial anthranilate synthetases are conserved.

The organization of genes for tryptophan biosynthesis has been studied in many eubacteria and eukaryotes (5, 6). Comparable information from archaebacteria remains limited. Meile et al. (18) recently completed the sequence of the *Methanobacterium thermoautotrophicum* gene cluster, finding open reading frames corresponding to all activities in the order *trpEGCFBAD*. We used an ordered set of cosmids bearing *Haloferax volcanii* DNA to transform tryptophan auxotrophs of this halophilic archaebacterium to prototrophy and localized all of 29 mutations to one of two regions of the genome (15). Sequencing of the first region (covering 10 mutations) revealed the overlapping genes *trpCBA*.

The 19 remaining mutations could be transformed to prototrophy with cosmid G203, 1,200 kbp away from the *trpCBA* cluster on the *H. volcanii* chromosome (2). Each mutation could also be transformed with ptrpE12, obtained by shotgun cloning wild-type DNA into our shuttle vector pWL102 (16). A 4-kbp *HindIII* subfragment was cloned into M13 vectors and sequenced (13, 19).

The region complementing anthranilate and indole-utilizing tryptophan auxotrophs contains four open reading frames (Fig. 1), each with significant sequence similarity to one of the tryptophan enzymatic domains defined by Hutter et al. (14).

The first gene of the cluster, *trpD*, encodes phosphoribosyltransferase (337 amino acids). All but a few of the conserved positions identified by Crawford and Milkman in an alignment of many eubacterial TrpD sequences are found in the *H. volcanii* sequence (5, 6), which shows 34 and 24% amino acid identity with the TrpD proteins of *Escherichia coli* and *Saccharomyces cerevisiae*, respectively.

H. volcanii trpF should encode N-phosphoribosylanthranilate isomerase (22 amino acids). Residues of presumed functional importance (6, 21) are largely conserved, and this protein is about equally similar to E. coli and S. cerevisiae homologs (30 and 32% amino acid identity, respectively).

The *H. volcanii trpE* gene which encodes the α subunit of anthranilate synthase (523 amino acids), does not begin with AUG. Overlapping the stop codon of the upstream *trpF* is an alternate start signal (GUGA). Meile et al. (18) also infer a GUG start for *M. thermoautotrophicum trpG*, and this gene too is preceded by *trpE*, but the extent of the overlap and the phase of the frameshift differ. The conserved segment between positions 52 and 55 of *trpE* (Leu-Leu-Glu-Ser), iden-

tified as a site for mutations affecting feedback inhibition by tryptophan in *Brevibacterium lactofermentum* (17) and *Salmonella typhimurium* (1), is found in the *H. volcanii* sequence. The halobacterial TrpE shows 29 and 30% amino acid sequence identity with *E. coli* and yeast enzymes, respectively, 34% identity with its *Methanobacterium* homolog, and comparable values with the homologous *pabB* and *phnA* genes of *E. coli* and *Pseudomonas aeruginosa* (Fig. 2). *H. volcanii* provides the only example of *trpE* embedded within a gene cluster—in all other organisms, this gene, encoding the first activity in the biochemical pathway, is the first gene of its cluster or is solitary (5, 6).

TrpG, the β subunit of anthranilate synthase (204 amino acids), is the smallest of the seven *H. volcanii* Trp proteins. It is 30 and 37% identical in sequence to *E. coli* and yeast homologs, respectively, and 38% similar to *M. thermoautotrophicum trpG*.

The G+C content of the *trpDFEG* genes (between 67 and 74 mol%) is similar to that of bulk *Haloferax* chromosomal DNA. Codons ending with C or G account for more than 90% of the fourfold-degenerate third positions of codons for alanine, glycine, proline, threonine, and valine. C is much preferred to G in the third position, except for codons with a C in the second position, as observed for the *H. volcanii hisC* locus (3).

All seven Trp enzymes of *H. volcanii* contain 17 to 20% acidic residues (aspartic acid and glutamic acid). Eisenberg and coworkers have argued that acidic residues stabilize proteins in the cytoplasm of halophilic archaebacteria, which is nearly saturated with KCl (7, 24).

The trpD, F, E, and G genes overlap by their stop and start codons (as do the genes of the trpCBA cluster). All four genes in the trpDFEG cluster are preceded (5 to 7 bp upstream of the ATG) by good potential ribosome-binding sequences: GGUGAU for trpD, GGAGG for trpF, AGG AGGU for trpE, and AGGAGG for trpG. The sequence of the 3' terminus of the H. volcanii small subunit rRNA is 3'-UCCUCCACUA (12). About 40 bp in front of the translation start of trpD is the sequence ATTTGTA, which resembles the archaebacterial box A promoter consensus [TTTA(A/T)A] (22, 25). A similar sequence (TTATGTA) is also found approximately 30 bp upstream from the start codon of trpCBA. No close approximations to these presumed promoter sequences are found within 100 bp upstream of any internal open reading frame in the trpDFEG cluster, but transcript analysis is required to exclude the

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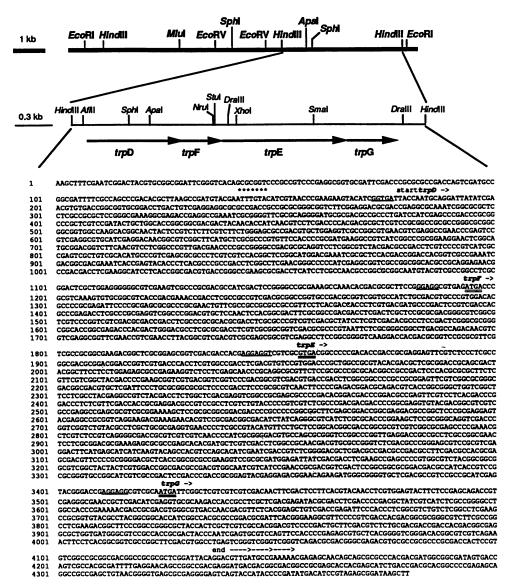


FIG. 1. The *trpDFEG* cluster. The restriction map of the chromosomal region cloned on plasmid ptrpE12 (see the text; a preliminary version of this map was presented in reference 15) and the sequence of a 4-kbp *Hind*III subfragment are shown. DNA sequences (both strands) for ordered sets of deletions in M13 were obtained by the dideoxy method using the Taquenase system (U.S. Biochemicals). Data bank searches were performed with FASTA (20) and Microgenie. Asterisks above the sequence indicate the putative promoter (22, 25), single underlining indicates presumptive ribosome binding sites, and double underlining shows inferred overlapping translational stops and starts. Dotted arrows under the sequence show direct repeats.

possibility that there are internal promoters, as in the *E. coli trp* operon (23).

The archaebacterial amino acid sequences for TrpD, TrpF, TrpE, and TrpG are as different from their eukaryotic (or indeed methanogen) homologs as from the eubacterial enzymes we have compared, and the eubacterial sequences are equally distant from the eukaryotic ones. Significant sequence identity seems confined to specific regions (presumably those essential to function), while variable sites have been saturated by mutations. This is similar to what we have seen with the *trpC*, *trpB*, and *trpA* comparisons described previously (15). Crawford and Milkman have made the same point in considering sequence divergence within the eubacteria alone (5, 6).

Both H. volcanii trp gene clusters are unusual in their

organization. These arrangements (trpDFEG and trpCBA) have little in common with the eukaryotic and eubacterial arrangements observed to date, except that the EG order and the BA order are often found in eubacteria. H. volcanii trp gene clusters also differ strikingly in organization from the single trpEGCFBAD gene cluster of M. thermoautotrophicum. In both cases, it seems reasonable to conclude that these clusters of very closely linked and often overlapping genes (without obvious individual consensus promoters) constitute operons (coordinately regulated and cotranscribed units), but this has not yet been directly shown. It is tempting to suppose that the last common ancestor of eubacteria and archaebacteria also had clustered and coordinately regulated trp genes, although it may be impossible to prove that once-isolated genes have not come together

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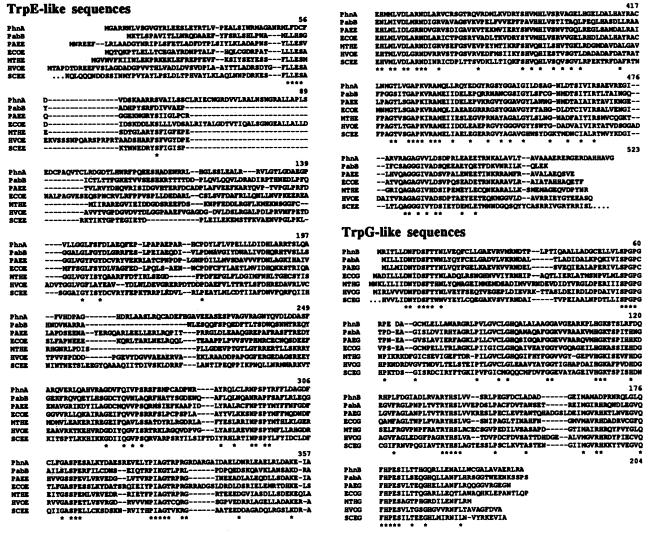


FIG. 2. Alignment of trpE, trpG, and related sequences. Inferred translation products of H. volcanii trpE and trpG (HVOE and HVOG) genes were aligned with trpE and trpG gene products from P. aeruginosa (PAEE and PAEG) (9), E. coli (ECOE and ECOG) (23), M. thermoautotrophicum (MTHE and MTHG) (18), and S. cerevisiae (SCEE and SCEG) (14) with the MULTALIN software of Corpet (4). Also aligned are the E. coli pabA and pabB and P. aeruginosa phnA and phnB gene products (8, 10). These two sets of genes code for a subunit of p-aminobenzoate synthase and a component of the phenazine pathway, respectively, which are homologs of anthranilate synthase subunits (5, 6). Asterisks mark residues shared by all trp gene products shown.

independently in several eubacterial and archaebacterial lineages to give rise to the patterns now observed. By either view, we may surely count the clustering of genes for related functions as one feature in which archaebacteria resemble eubacteria.

Nucleotide sequence accession number. The sequences described in this report will appear in the EMBL GenBank with the accession number M83788.

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